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Alterations in mRNA levels of two neurotrophins and their receptors were characterized in a rat model of traumatic brain injury with in situ hybridization in order to investigate their role in neuroprotection. Complimentary increases in brain-derived neurotrophic factor (BDNF) and its receptor trkB were observed in the dentate gyrus of the hippocampal formation, an area that is selectively resistant to neuronal degeneration following experimental brain trauma. Conversely, neurotrophin-3 (NT-3) and its receptor, trkC, decreased in this same brain region. In the most severely damaged part of the brain, the cortical lesion site, BDNF mRNA levels never increased over control values, and were significantly decreased by 72 h post-injury. These data support a role for BDNF/trkB, but not NT-3/trkC interactions in neuroprotection following experimental brain trauma.

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Ramsey Hsu Jan. 8, 1998

PI - Signature Date

4. Table of Contents

5. Introduction

The long term objective of this study is to improve functional outcome following traumatic brain injury (TBI) by enhancing mechanisms which are neuroprotective. This study investigates the role of neurotrophic factors in attenuating neuronal injury after brain trauma. Neurotrophic factors are a family of structurally related polypeptides which have been shown to play a critical role during neuronal development and appear to mediate a protective response to trauma and disease in mature animals (Hefti et al., 1989; Mattson & Scheff, 1994). One of the most abundantly expressed neurotrophic factors in the central nervous system (CNS), brain-derived neurotrophic factor (BDNF), is of particular interest in regard to recovery following injury, because its expression is greater in adults than in developing animals (Hofer et al., 1990; Maissonpierre et al., 1990), and because it appears that BDNF may function in an autocrine or paracrine fashion rather than as a target-derived molecule (Acheson et al., 1995; Lucidi-Phillipi and Gage, 1993; Seroogy et al., 1994; Wetmore et al., 1994). Previous studies have demonstrated that administration of BDNF following glucose deprivation (Cheng & Mattson, 1994), excitoxicity (Ballarin et al., 1994), and ischemia (Beck et al., 1993; Tsukahara et al., 1994) attenuated cell death. In addition, following ischemic injury, mRNA for BDNF was strongly and selectively elevated in cell populations that were resistant to cell death (Lindvall et al., 1992).

In the adult CNS, BDNF is the most prevalent member of the family of neurotrophic factors, which also includes nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and neurotrophin-5 (NT-5). The trophic properties of these neurotrophins are mediated through interaction with their receptors, which are transmembrane protein-kinases (trks) (Jing et al., 1992). The widely expressed trkB protein is the high affinity receptor for BDNF and NT-4 (Barbacid, 1994; Lindsay, 1994). Activation of trkB involves ligand binding by the neurotrophin, receptor dimerization, autophosphorylation, and activation of tyrosine residues on various intracellular substrates (Jing et al., 1992). These intracellular substrates serve as signals for survival, proliferation, differentiation, and synaptogenesis, as well as other forms of neural plasticity (Lindholm et al., 1994; Snider, 1994).

The purpose of the first year of the grant was to investigate the expression of BDNF and trkB mRNA in a well-characterized rat model of traumatic brain injury, the lateral fluid percussion model (McIntosh et al., 1989; Hicks et al., 1993, 1996; Soares et al., 1995). In addition, because recent studies suggest that BDNF and NT-3 may have opposing roles and be differentially regulated during development (McAllister et al., 1997), alterations in the mRNA levels of NT-3 and its receptor, trkC, were also characterized in some studies.

6. Body

Methods

Male Sprague-Dawley rats (325-350g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) 10 min after receiving 0.15 ml of atropine (0.4 mg/ml, i.m.), and placed in a stereotaxic frame. The scalp and temporal muscles were reflected, and a stainless-steel screw was secured to the skull 1 mm anterior to bregma. A hand-held trephine with a 4.9 mm diameter was used to make a craniotomy, which was centered between bregma and lambda, 3 mm lateral to the sagittal suture. A Luer-loc hub was rigidly fixed with dental cement to the craniotomy. Experimental brain injury of moderate severity (2.0 - 2.1 atm) was induced in the anesthetized animals (n=20), using the lateral FP brain injury model. This model is well-characterized and has been previously described in detail (Cortez et al., 1989; Hicks et al., 1996; McIntosh et al., 1989). Following FP injury, rats were euthanized at 1, 3, 6, 24, or 72 h, in order to assess the acute response of BDNF, NT-3, trkB and trkC to the injury. Additional animals (n=15; 3/survival period) underwent anesthesia and surgery but were not injured (sham treatment).

The effects of mild injury were also investigated in another group of animals. The procedure was identical to that described in the previous paragraph except for the following:

1. The experimental brain injury was of mild severity (1.0 atm).
2. Based on the data obtained following a moderate injury, which showed maximal changes at 3 and 6 h post-injury, injured (n=4) and control animals (n=3) were studied for these 2 time points.

After the appropriate survival times, the rats were deeply anesthetized with an overdose of sodium pentobarbital and decapitated. Brains were rapidly removed and frozen over dry ice. Tissue sections through the hippocampus were cut in the coronal plane at 10 μm in a cryostat, thaw-mounted onto Superfrost Plus (Curtin Matheson Scientific) glass slides, and stored at -20°C until processing for hybridization. Adjacent sections throughout the hippocampus of animals from the various injury and sham groups were processed for the in situ hybridization localization of mRNAs for BDNF, NT-3, trkB and trkC as previously described (Gall et al., 1992; Seroogy et al., 1994; Seroogy and Herman, 1997). The cRNA probes were prepared by in vitro transcription from linearized cDNA constructs with the appropriate RNA polymerase in the presence of ^{35}S-UTP. Hybridization was conducted at 60° C for 18-24 h with the ^{35}S-labeled cRNA at a concentration of $1x10^6$ cpm/50 μl/slide. Following post-hybridization washes and ribonuclease treatment, the sections were air-dried and exposed to β-Max Hyperfilm (Amersham) for 14-18 days at room temperature for generation of film autoradiograms. After autoradiographic film development, the sections were dipped in NTB2 nuclear track emulsion (Kodak; 1:1 in H_2O), air-dried, and exposed in light-tight boxes at 4°C for 4-6

weeks. After autoradiographic development of the emulsion, the sections were counterstained with Cresyl violet, coverslipped in D.P.X. mounting medium (Fluka), and analyzed with a Nikon Optiphot-2 microscope equipped with brightfield and darkfield optics. Cells were considered labeled if the density of reduced silver grains overlying the perikarya was at least 10-fold greater than background. Control sections that had been treated with ribonuclease A (45°C for 30 min) before hybridization or processed for hybridization with appropriate sense-strand riboprobes (see Gall et al., 1992) were devoid of specific labeling.

Film autoradiograms were analyzed with Image 1.60 software (NIH) to measure the density of hybridization for the neurotrophin and trk mRNAs in various hippocampal subfields (dentate gyrus, CA1 and CA3) and in various cortical subfields (injured cortex, superficial and deep layers of the adjacent cortex, superficial and deep layers of the opposite cortex, and the bilateral piriform cortex. At least 3 sections were analyzed per animal. All measurements are expressed as the mean values plus or minus the standard error of the mean (SEM). The effects of treatment, survival time, and the interaction were analyzed with a two-way analysis of variance (ANOVA) in each hippocampal subfield for the side of the brain ipsilateral to the injury, contralateral to the injury, and for the bilateral sham data. Bonferonni post-hoc analyses were used for pairwise comparisons with a significance set at $P< 0.05$.

Results and Discussion

BDNF and NT-3 mRNA in the Hippocampus. Hybridization for BDNF mRNA was present in the granule cell layer of the dentate gyrus (stratum granulosum) and in regions CA1-CA3 of the hippocampus in the control (sham treatment) animals (Fig. 1A), similar to previous reports in normal, uninjured rats (Enfors et al., 1990; Isackson et al., 1991). Unilateral FP injury resulted in a marked bilateral increase in the expression of BDNF mRNA in the dentate gyrus granule cell layer, which peaked at 3 h and remained above control levels for up to 72 h after injury (Fig. 1B-D). Densitometric measurements of film autoradiograms demonstrated that BDNF mRNA hybridization was significantly increased in the granule cell layer at all post-injury time points ($P<0.001$; Fig. 2A). Expression of BDNF mRNA was also significantly elevated bilaterally in the hippocampal CA3 region at 1, 3 and 6 h after FP injury ($P<0.001$), but returned to control levels by 24 h (Fig. 1B-D). Again, the most pronounced increase in labeling in the CA3 region was evident at 3 h after injury (Fig. 2B). No changes in expression of BDNF mRNA occurred in the CA1 region of the hippocampus following FP injury (Fig. 2C).

In the control, sham-injured animals the most prominent expression of NT-3 mRNA was localized to the dentate gyrus granule cell layer (Fig. 1E). Labeled cells were also present in regions CA2 and extreme medial CA1 of the hippocampal pyramidal cell layer, as well as infrequently scattered throughout the dentate gyrus hilus and hippocampal molecular layers (Fig. 1E). This distribution is in good agreement with previous descriptions in normal rats (Enfors et al., 1990 and Gall and Lauterborn, 1992). Following unilateral FP injury, hybridization for NT-3 mRNA was decreased bilaterally

in the dentate gyrus granule cell layer at the 6 and 24 h survival times (Fig. 1G). By 72 h post-injury, hybridization levels had returned to near-control (sham injury) levels (Fig. 1H). Quantitative measurements of film autoradiograms confirmed that NT-3 mRNA hybridization was significantly reduced in the granule cells at both 6 and 24 h after FP injury ($P<0.001$; Fig. 3), compared to sham controls. Although not analyzed densitometrically, visual inspection of NT-3 mRNA hybridization in CA2 and medial CA1 indicated no apparent change in expression at any of the survival times post-injury, compared to sham controls.

The present results demonstrate that FP brain injury induces pronounced alterations in the expression of neurotrophin mRNAs in the hippocampus. Levels of BDNF mRNA were substantially increased post-injury in both the dentate gyrus granule cell and CA3 pyramidal cell layers. In contrast, expression of NT-3 mRNA was transiently decreased in the dentate gyrus, and the response was delayed relative to the early change in BDNF. Thus, traumatic brain injury differentially modulates neurotrophin gene expression in the hippocampus, in patterns and directions similar to findings in other brain injury paradigms including ischemia and seizures (Gall, 1993; Gall & Lauterborn, 1992; Lindvall et al., 1992; 1994).

The bilateral alterations in BDNF and NT-3 expression are in contrast to the gross morphological and histological damage which has been primarily identified in hippocampal regions ipsilateral to the impact site (Cortez et al., 1989; Hicks et al., 1996). However, they are consistent with more subtle changes, such as the bilateral loss of hilar neurons (Lowenstein et al., 1992), and bilateral alterations in the expression of immediate-early genes and tumor necrosis factor-α (Fan et al., 1996; Raghupathi and McIntosh, 1996), which have been observed following lateral FP injury.

The significance of the alterations in BDNF and NT-3 expression on cell survival is unclear. The dentate gyrus showed the greatest increase in BDNF compared to control values and cells in this region are selectively resistant to cell death following lateral FP injury (Cortez et al., 1989; Hicks et al., 1996; Lowenstein et al., 1992). However, BDNF expression was also elevated bilaterally in the hippocampal CA3 region, which contains numerous injured neurons on the side ipsilateral to the impact (Cortez et al., 1989; Hicks et al., 1996). Numerous previous studies have supported the hypothesis that BDNF is neuroprotective following injury (Ballarin et al, 1991; Cheng and Mattson, 1994; Hayes et al., 1995; Lindvall et al., 1992, 1994; Tsukahara et al., 1994), whereas others have found no trophic effect (Rudge et al., 1995), or an actual increase in neuronal death (Koh et al., 1995) with BDNF treatment. Although further studies are necessary to clarify the role of BDNF following injury, one hypothesis is that it is the *amount* of BDNF available that is critical for promoting cell survival. The functional consequences of the concurrent decrease in NT-3 expression in the same cells (stratum granulosum) marked by the BDNF increase, also remain unknown. It is possible that whereas optimal neurotrophin levels may promote survival, insufficient or excessive levels may exacerbate neuronal loss. Moreover, injury-induced alterations in levels and functional states of appropriate

neurotrophin receptors may also be important determinants of resulting neurotrophic functions.

This study has been published and is attached as Appendix I.

trkB and trkC mRNA in the hippocampus. Hybridization for trkB and trkC mRNA was present in the granule cell layer of the dentate gyrus (stratum granulosum) and in regions CA1 and CA3 of the hippocampus in the control (sham treatment) animals, similar to previous reports in uninjured rats (Fryer et al., 1996; Klein et al., 1990; Middlemas et al., 1991). In each hippocampal subfield, sham trkB and trkC mRNA expression remained stable over time. Following FP injury, elevations in trkB mRNA were visible in the dentate gyrus at 3 and 6 h post-FP injury, but not at 1, 24 or 72 h. Densitometric measurements of film autoradiograms confirmed that trkB mRNA was significantly increased in both the right and left stratum granulosum compared to sham controls at 3 h ($P< 0.005$) and at 6 h ($P < 0.05$), (Fig. 4A). In the CA1 and CA3 regions, time-dependent changes in trkB mRNA were not observed. However, there were differences across groups after FP injury, with a small but significant decrease in the left CA1 region ($P< 0.005$) and in the left CA3 region ($P< 0.001$) compared to shams (Fig. 4B).

Hybridization for trkC mRNA was decreased bilaterally in the dentate gyrus granule cell layer at the 24 h survival time following FP injury compared to the 24 h sham controls. This decrease was not observed at earlier times or for the 72 h survival period. Quantitative measurements of film autoradiograms confirmed that trkC expression was significantly reduced in the stratum granulosum at 24 h after FP injury ($P< 0.02$), compared to sham controls (Fig. 5A). FP injury also produced small but significant decreases in trkC mRNA in the ipsilateral CA1 ($P < 0.005$) and ipsilateral CA3 ($P < 0.001$) regions of the hippocampus (Fig. 5B), but these alterations were not time-dependent.

The major findings of this study are that trkB mRNA was significantly increased at 3 h (41% above sham levels) and at 6 h (38% above sham values), and trkC mRNA was significantly decreased at 24 h (17% below sham values) in the bilateral dentate gyrus following lateral FP brain injury. Time-dependent changes were not observed in CA1 and CA3, but there were significant differences between injured and sham animals. The ipsilateral CA1 region showed a minor decreases in trkB (11% below sham values) and trkC (6% below sham values) hybridization. The ipsilateral CA3 region also demonstrated a decrease in trkB mRNA (15% below sham values) and trkC mRNA (9% below sham values) levels. The decreases in hybridization in the CA3 region may be related to the rapid neuronal degeneration that has been demonstrated in this structure following FP injury (Hicks et al., 1996; Soares et al., 1995; Dietrich et al., 1994).

The alterations in trkB and trkC mRNA that we observed in the dentate gyrus are in general agreement with previous investigations utilizing other models of CNS injury. Induction of seizures following kindling caused a rapid and transient elevation in trkB

mRNA, which peaked at 30 min and tapered off to near control values by 4 h (Merlio et al., 1993). No changes were observed in trkC mRNA after seizures. An ischemic insult caused an increase in trkB mRNA in the dentate gyrus at 2 h post-injury (Merlio et al., 1993) , which is very similar to the temporal profile that we observed after FP injury. A similar time course was also observed after a focal, mechanical injury to one side of the brain, which produced a unilateral increase in both trkB and trkC mRNA in the dentate gyrus between 2- 4 hr post-injury (Mudo et al., 1993). In contrast to Mudo et als. study, we observed significant alterations bilaterally in the dentate gyrus. Recent studies have demonstrated that subtle neurological sequelae are often present bilaterally following a lateral FP injury (Hicks et al., 1997; Raghupathi et al., 1996; Fan et al., 1996), whereas overt histopathological changes are primarily restricted to the side of the brain ipsilateral to the impact site (Cortez et al., 1989; McIntosh et al., 1989; Hicks et al., 1996; Soares et al., 1995).

In a previous study, BDNF mRNA increased between 174-235% and NT-3 mRNA decreased between 73-81% compared to sham controls after FP injury (Hicks et al., 1997). Thus, the increase in trkB mRNA and the decrease in trkC mRNA in the dentate gyrus after FP injury was much less robust than for their respective ligands. Nevertheless, the complementary increases in BDNF mRNA and the full-length trkB receptor mRNA levels, suggests that this NTF/trk signal transduction pathway may be greatly enhanced in the dentate gyrus after FP injury. This is an important observation because chronic exposure to BDNF has been shown to down-regulate trkB mRNA and protein in vivo (Frank et al., 1996; Knusel et al., 1997).

The functional significance of the increase in BDNF/trkB mRNA and the decrease in NT-3/trkC mRNA in the dentate gyrus following FP injury is unknown. Originally neurotrophins were believed to be important for their role in selective neuronal survival (Johnson and Oppenheim, 1994). This role may also be important following FP injury. BDNF/trkB signal transduction may have a neuroprotective effect on the granule cells of the dentate gyrus following FP injury, as these cells are selectively resistant. However, CA1 cells are also resistant to FP-induced degeneration, and increases in BDNF/trkB were not observed in this region of the hippocampus (Hicks et al., 1996). NT-3/trkC signal transduction has also been found to have neuroprotective effects in some models of neuronal injury (Lindsay, 1996), but that does not appear to be the case following FP injury as both neurotrophin and receptor mRNA levels were decreased in the dentate gyrus (Hicks et al., 1997).

NTF/trk interactions have also been associated with synaptogenesis and neural plasticity (Cabelli et al., 1997). Blocking trkB receptors interfered with the normal development of ocular dominance columns in the visual cortex (Cabelli et al., 1997). Removal of facial vibrassae in mice during development resulted in a decrease in BDNF mRNA in the corresponding cortical barrel region, but an increase in the contralateral barrel region (Singh et al., 1997). A recent paper proposes that BDNF and NT-3 may have antagonistic actions on dendritic growth in cortical neurons (McAllister et al., 1997). A similar relationship may exist in the hippocampus, and a decrease in NT-3/trkC

mRNA may further amplify the effects of increased levels of BDNF/trkB mRNA on neural plasticity.

A manuscript entitled "Expression of trkB and trkC mRNA is altered in rat hippocampus after experimental brain trauma" by R.R. Hicks, L. Zhang, H.S. Dhillon, M.R. Prasad, and K.B. Seroogy is in progress. A draft of the manuscript has been attached as Appendix II.

BDNF and trkB mRNA in the Cortex. Hybridization for BDNF mRNA in the neocortex and piriform cortex in the control animals was similar to previous reports in normal, uninjured rats (Enfors et al., 1990; Isackson et al., 1991). Unilateral FP injury resulted in a decrease in expression of BDNF mRNA in the injured cortex, which reached statistical significance at 72 h post-injury ($P<0.01$), (Fig. 6). In contrast to the decrease in the injured cortex, BDNF mRNA levels were significantly increased in the bilateral piriform cortex at 3, 6, and 24 h (Fig.7), and in the superficial layers of neocortical regions adjacent to the injured cortex at 3 h post-injury (Fig. 8A). Time-dependent alterations were not observed in the deep layers of the adjacent cortex (Fig. 8B), or the superficial or deep layers of the contralateral (opposite) cortex (Fig. 9A and B). However, these brain regions demonstrated small, but significant increases in BDNF mRNA following FP injury compared to control animals when all of the data was combined for each group ($P<0.001$).

Optical density measurements of trkB mRNA in the neocortex and piriform cortex have also been completed and analysis is in progress. Preliminary results suggest that the response to injury appears less robust than for that of BDNF mRNA. Of the four areas analyzed (deep and superficial layers of the adjacent and opposite cortex), only the superficial layers of the adjacent cortex demonstrate a significant time-dependent increase compared to sham animals ($P< 0.003$ for injured vs. sham animals 3 h post-injury). The other cortical brain areas are currently being analyzed for trkB hybridization and the findings from this study will be written up and submitted for publication in the Spring of 1998.

Alterations in Neurotrophins and trks following mild injury. This work is in progress. Tissue from all of the animals has been sectioned and is being processed for in situ hybridization of BDNF, NT-3, trkB, and trkC mRNA at this time.

7. Conclusions

1. Lateral FP brain injury leads to acute, widespread alterations in BDNF, NT-3, trkB and trkC mRNA levels in the hippocampus and cortex. These alterations support a role for neurotrophins and their receptors in secondary events associated with traumatic brain injury.

2. Induction of BDNF mRNA is not associated with neuronal injury in the cortex.

3. Induction of BDNF and trkB mRNA may be neuroprotective as the brain regions with the greatest increases (the dentate gyrus granule cell layer and piriform cortex) are selectively resistant to neuronal injury.

4. Acute increases in BDNF mRNA are associated with acute increases in the trkB receptor following lateral FP injury. This is important because chronic infusion of BDNF has been associated with down-regulation of the trkB receptor. Acute delivery of BDNF following traumatic brain injury may be more effective for BDNF/trkB signal transduction.

5. NT-3 and trkC mRNA decrease in brain regions that show an increase in BDNF and trkB mRNA. The antagonistic expression of these neurotrophins may serve to amplify the effects of the BDNF/trkB signal transduction pathways.

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Figure 1. Prints of film autoradiograms showing expression of BDNF (A-D) and NT-3 (E-H) mRNAs in coronal sections from control (sham) rats (A,E), and from rats subjected to moderate lateral FP brain injury with 3 (B,F), 24 (C,G), and 72 h (D,H) survival periods. Note the increased hybridization for BDNF mRNA bilaterally in the dentate gyrus granule cell layer (stratum granulosum; sg) at all survival times following injury (B-D), and in the hippocampal CA3 region at the 3 h post-injury time point (B). In contrast, NT-3 mRNA levels are decreased bilaterally in the dentate gyrus at 24 h post-injury (G). Arrowheads in appropriate panels indicate the cell layers and survival times which differ significantly from the sham controls (see quantification in Figs. 2 and 3). Scale bar = 500 μm.

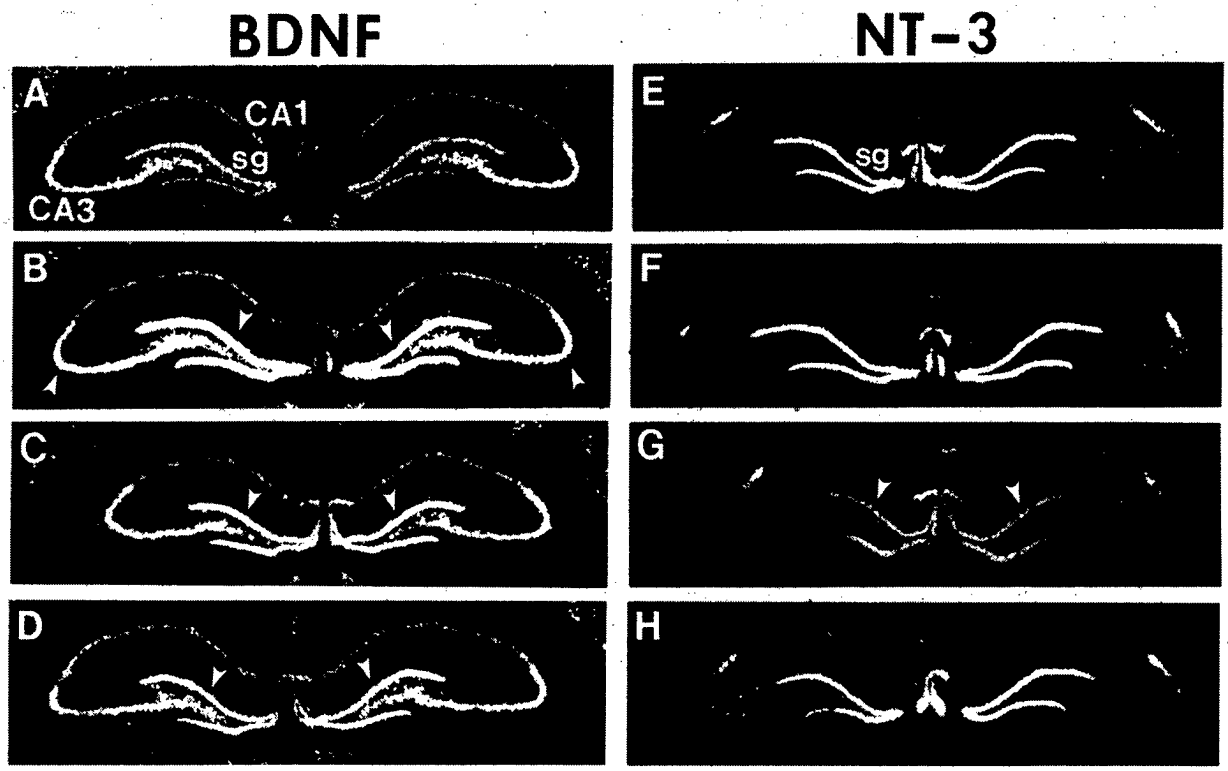


Figure 2. Graphs showing corrected optical density (O.D.) measurements of hybridization for BDNF mRNA in the dentate gyrus stratum granulosum (A), hippocampal CA3 (B), and hippocampal CA1 (C) regions over time following lateral FP injury. Note the significant increase in BDNF mRNA expression in the dentate gyrus granule cell layer at all post-injury times (A), and in the hippocampal CA3 region at 1, 3, and 6 h following injury (B), compared to the sham treatment group (*P<0.001). Lateral FP injury did not alter BDNF mRNA levels in the hippocampal CA1 region at any of the survival times (C). Values represent mean ± S.E.M.

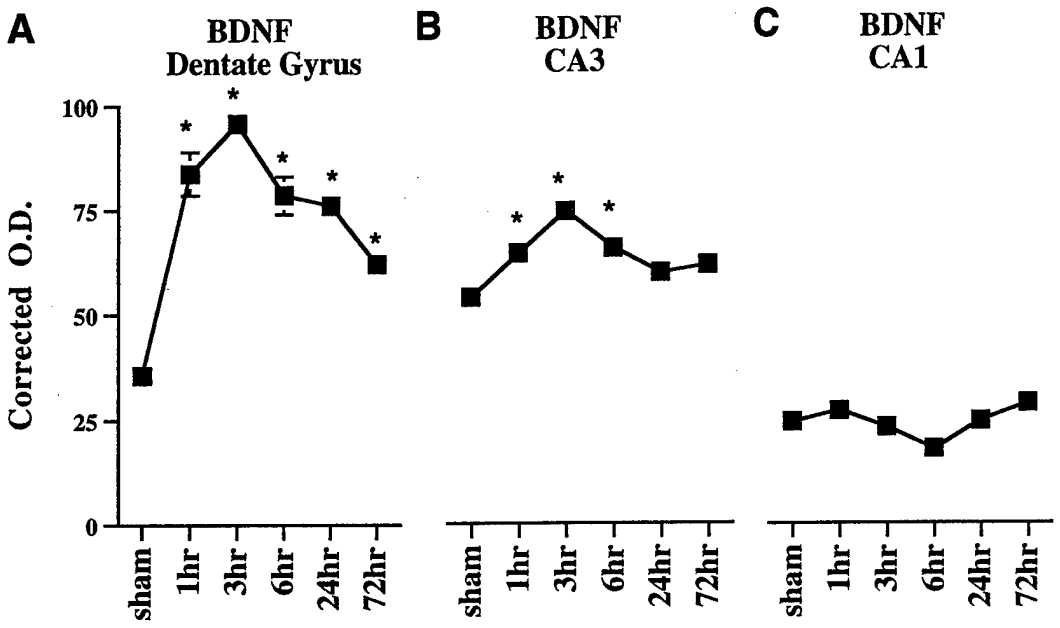


Figure 3. Graphs showing corrected optical density (O.D.) measurements of NT-3 hybridization in the dentate gyrus granule cell layer over time following lateral FP injury. Note the significant decline in NT-3 mRNA expression at 6 and 24 h after injury compared to the sham treatment group (*P<0.001). Values represent mean ± S.E.M.

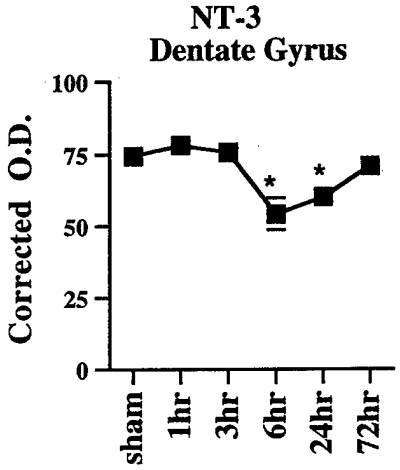
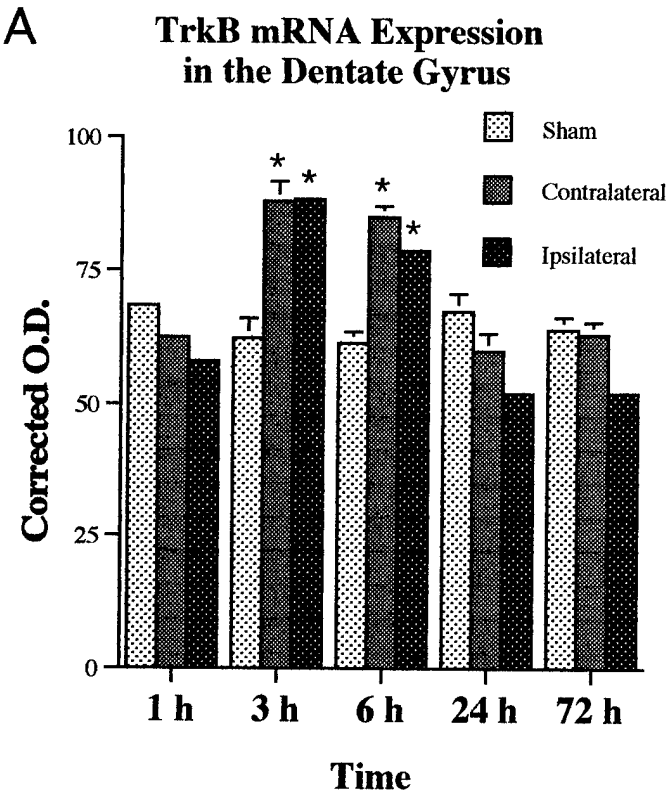


Figure 4. Graphs show trkB gene expression in sham, ipsilateral and contralateral hippocampus at 1, 3, 6, 24, and 72 h post-FP injury in dentate gyrus (A) and for all times in the CA1 and CA3 regions (B). trkB mRNA levels were quantitated by image analysis of film autoradiograms developed following in situ hybridization and then plotted as mean ± SEM. *Indicates mRNA levels were significantly different from sham levels.



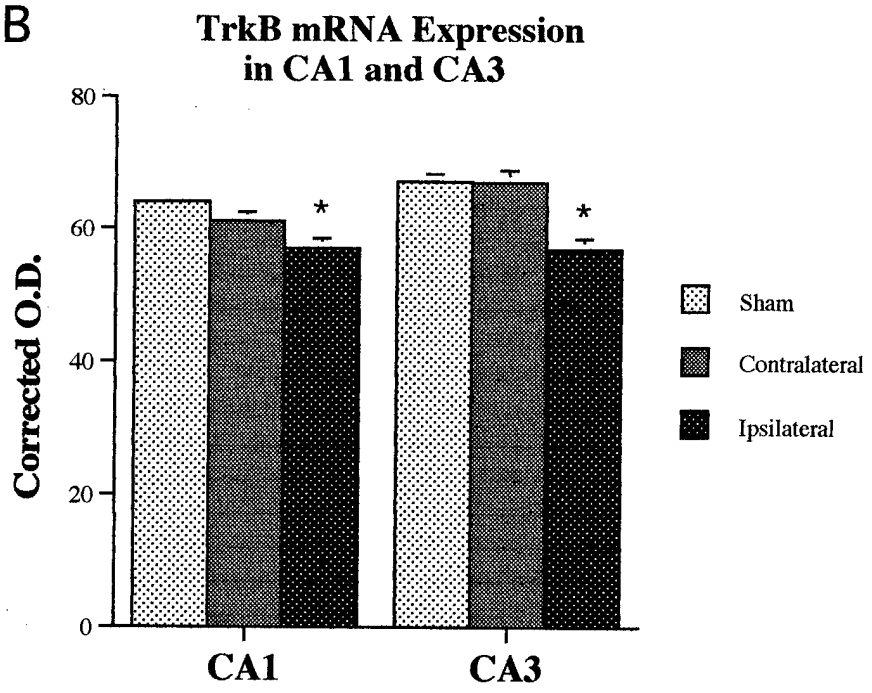


Figure 5. Graphs show trkC gene expression in sham, ipsilateral and contralateral hippocampus at 1, 3, 6, 24, and 72 h post-FP injury in dentate gyrus (A) and for all times in the CA1 and CA3 regions (B). trkC mRNA levels were quantitated by image analysis of film autoradiograms developed following in situ hybridization and then plotted as mean ± SEM. *Indicates mRNA levels were significantly different from sham levels.

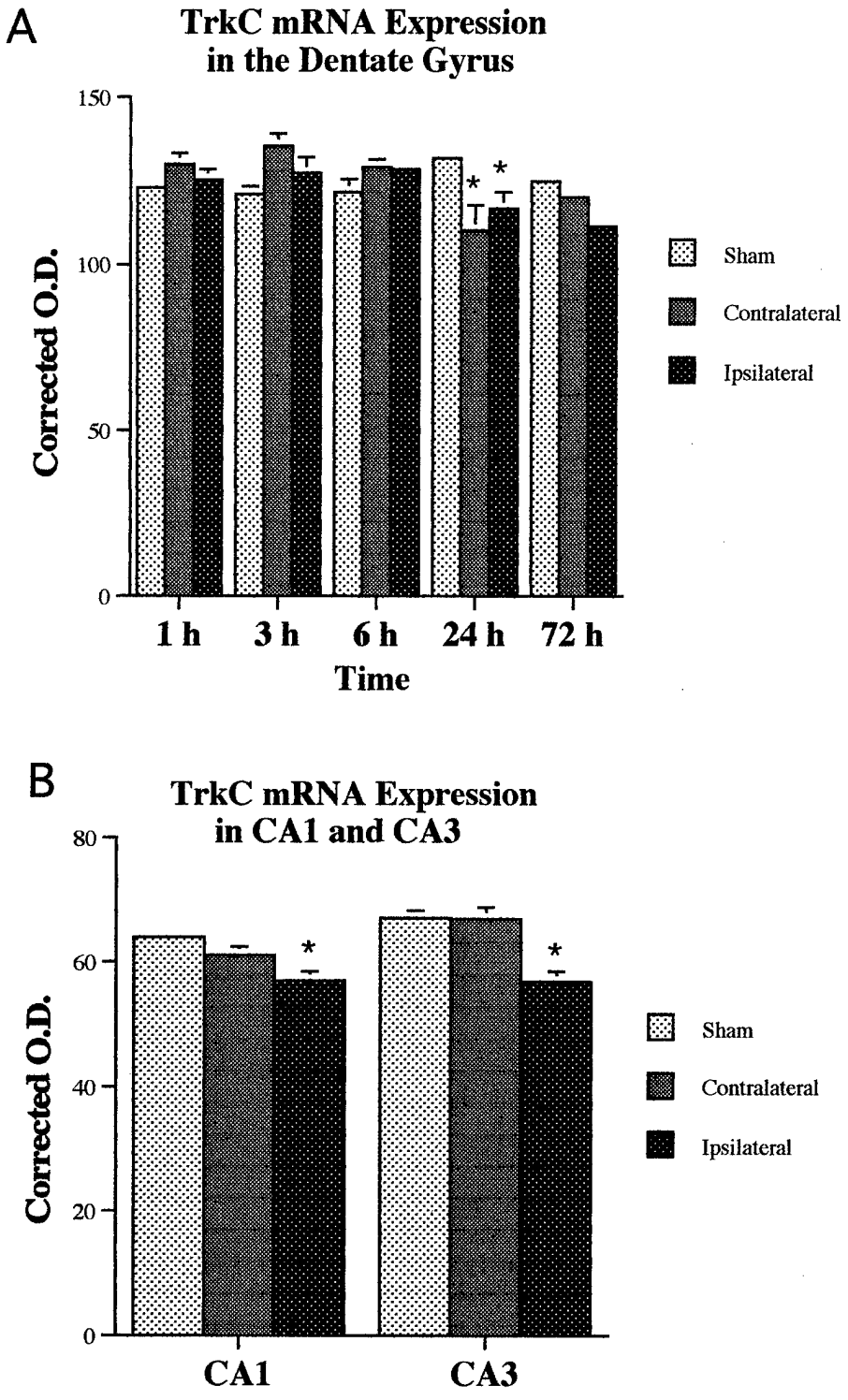


Figure 6. Graphs showing corrected optical density (O.D.) measurements of BDNF hybridization in the injured cortex over time following lateral FP injury. Note the significant decline in BDNF mRNA expression at 72 h after injury compared to the sham treatment group (*P<0.01). Values represent mean ± S.E.M.

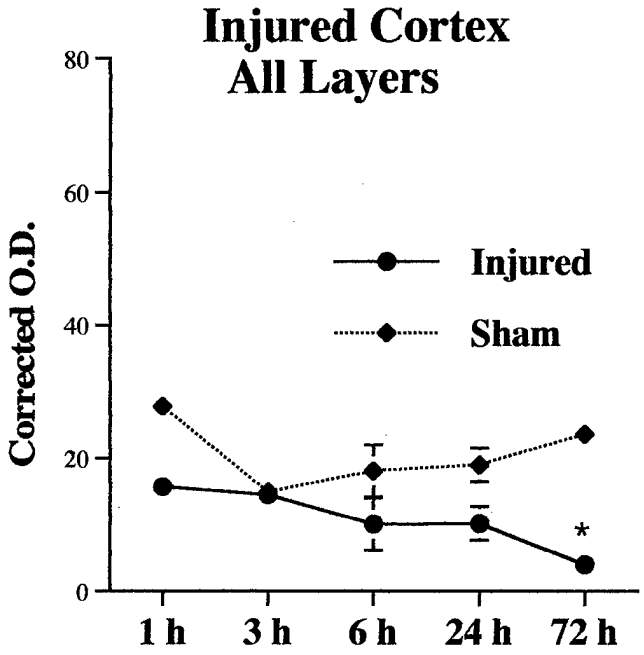


Figure 7. Graph shows corrected optical density (O.D.) measurements of hybridization for BDNF mRNA in the bilateral piriform cortices over time following lateral FP injury. Note the significant increase in BDNF mRNA expression at 3, 6 and 24 h following injury compared to the sham treatment group (*P<0.001). Values represent mean ± S.E.M.

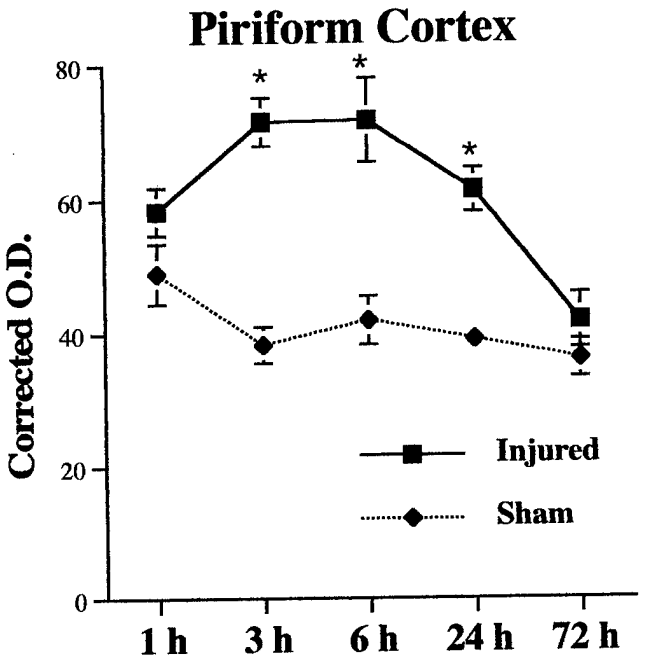


Figure 8. Graphs showing corrected optical density (O.D.) measurements of BDNF hybridization in the superficial (A) and deep (B) layers of the adjacent cortex (AC) over time following lateral FP injury. Note the significant increase in BDNF mRNA expression at 3 h after injury in the superficial layers of the AC compared to the sham treatment group (*P<0.01). Values represent mean ± S.E.M.

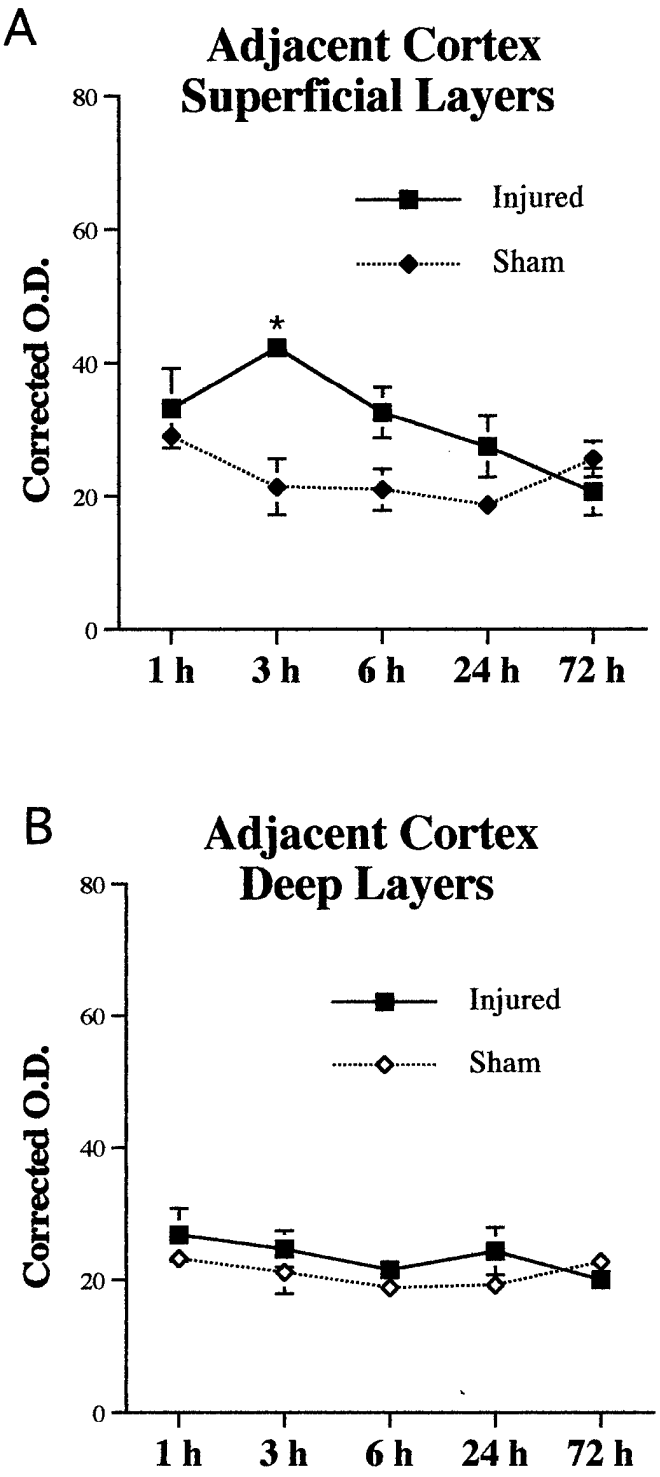
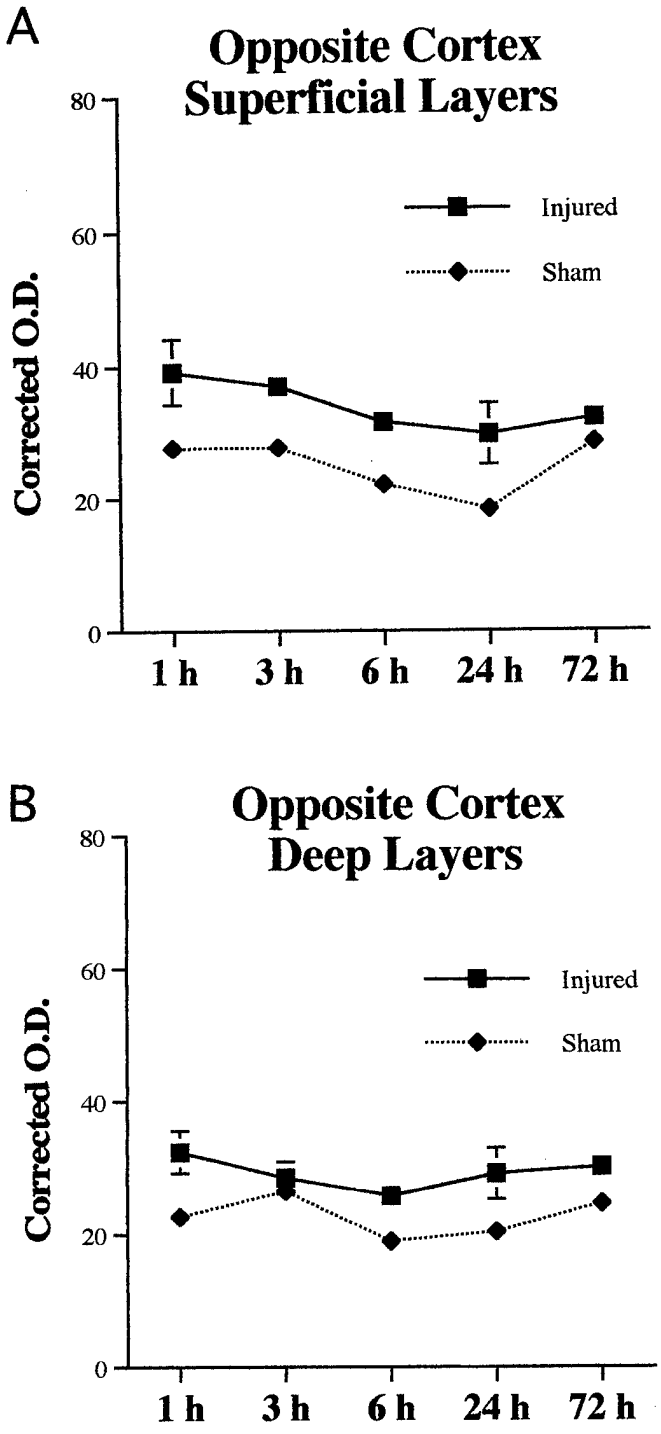


Figure 9. Graphs showing corrected optical density (O.D.) measurements of BDNF hybridization in the superficial (A) and deep (B) layers of the opposite cortex (OC) over time following lateral FP injury. Time-dependent alterations were not observed in the OC following lateral FP injury. Values represent mean ± S.E.M.



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Short communication

Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma

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Abstract

Previous studies have suggested that the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are neuroprotective or neurotrophic for certain subpopulations of hippocampal neurons following various brain insults. In the present study, the expression of BDNF and NT-3 mRNAs in rat hippocampus was examined after traumatic brain injury. Following lateral fluid percussion (FP) brain injury of moderate severity (2.0–2.1 atm) or sham injury, the hippocampi from adult rats were processed for the in situ hybridization localization of BDNF and NT-3 mRNAs using ^{35}S-labeled cRNA probes at post-injury survival times of 1, 3, 6, 24 and 72 h. Unilateral FP injury markedly increased hybridization for BDNF mRNA in the dentate gyrus bilaterally which peaked at 3 h and remained above control levels for up to 72 h post-injury. A moderate increase in BDNF mRNA expression was also observed bilaterally in the CA3 region of the hippocampus at 1, 3, and 6 h after FP injury, but expression declined to control levels by 24 h. Conversely, NT-3 mRNA was significantly decreased in the dentate gyrus following FP injury at the 6 and 24 h survival times. These results demonstrate that FP brain injury differentially modulates expression of BDNF and NT-3 mRNAs in the hippocampus, and suggest that neurotrophin plasticity is a functional response of hippocampal neurons to brain trauma. © 1997 Elsevier Science B.V.

Keywords: Traumatic brain injury; Lateral fluid percussion; Brain-derived neurotrophic factor; Neurotrophin-3; Hybridization, in situ; Neuronal plasticity

Secondary or delayed injury processes that begin to develop within minutes and continue to develop for hours after traumatic brain injury can contribute to irreversible tissue damage [3]. Although the sequence and timing of these processes are largely unknown, they are thought to be initiated by the release of neurotransmitters such as excitatory amino acids [8,23,35,37] and acetylcholine [14], and by the subsequent activation of neurotransmitter receptors, including NMDA-receptor subtypes, muscarinic cholinergic receptors, and opioid receptors [6,16,29,38]. An increase in intracellular calcium and the subsequent stimulation of calcium-dependent enzymatic activities are implicated mediators in some of the neurotransmitter-induced generation of secondary injury factors [6,10,16,19,45].

Several studies indicate that activation of excitatory amino-acid receptors in neurons can also result in the induction of neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [34,47] (for review, see [26]). Accordingly, increased gene expression for BDNF and NGF in the brain has been observed in several models of central nervous system injury, such as ischemia and seizures, where excitatory amino-acid receptors are implicated in the pathogenesis [12,21,27,46]. In contrast to the up-regulation of NGF and BDNF mRNAs, down-regulation of neurotrophin-3 (NT-3) mRNA has also been observed with cerebral ischemia and seizures [12,27]. It has been suggested that these neurotrophins may provide neuroprotection by playing a role in the maintenance and survival of neurons after traumatic brain injury [31,33]. Therefore, it is important to characterize the spatial and temporal patterns and levels of neurotrophic factor expression after experimental brain injury.

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Relatively few studies have examined alterations in the neurotrophin family of trophic factors after traumatic brain injury. Recent reports have, however, demonstrated increases of BDNF and NGF mRNAs and NGF protein in cortical areas after cortical contusion brain injury [5,15,48]. The present study characterized the changes in BDNF and NT-3 mRNAs in the hippocampus after lateral fluid percussion (FP) brain injury, another established model of traumatic brain injury. The hippocampus was of particular interest because of its prominent expression of and responsiveness to neurotrophins [2,13,22,25], its vulnerability to neurodegeneration subsequent to various brain insults [41], and its role in learning and memory dysfunction following FP injury [18,44].

Male Sprague-Dawley rats (325–350 g) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) 10 min after receiving 0.15 ml of atropine (0.4 mg/ml i.m.), and placed in a stereotaxic frame. The scalp and temporal muscles were reflected, and a stainless-steel screw was secured to the skull 1 mm anterior to bregma. A hand-held trephine with a 4.9 mm diameter was used to make a craniotomy, which was centered between bregma and lambda, 3 mm lateral to the sagittal suture. A Luer-loc hub was rigidly fixed with dental cement to the craniotomy. Experimental lateral FP brain injury of moderate severity (2.0–2.1 atm) was induced in the anesthetized animals ($n = 20$) using a well-characterized model that has been previously described in detail [20,32]. Following FP injury, rats were allowed to survive for 1, 3, 6, 24 or 72 h before euthanasia, in order to assess the acute response of the neurotrophins to the injury. A subset of animals ($n = 4$; 3 h survival period) underwent anesthesia and surgery but were not injured (sham treatment).

After the appropriate survival times, the rats were deeply anesthetized with an overdose of sodium pentobarbital and decapitated. Brains were rapidly removed and frozen over dry ice. Tissue sections through the hippocampus were cut in the coronal plane at 10 μm in a cryostat, thaw-mounted onto Superfrost Plus (Curtin Matheson Scientific) glass slides, and stored at -20°C until processing for hybridization. Adjacent sections throughout the hippocampus of animals from the various injury and sham groups were processed for the in situ hybridization localization of mRNAs for BDNF and NT-3 as previously described [11,42,43]. The cRNA probes were prepared by in vitro transcription from linearized cDNA constructs with the appropriate RNA polymerase in the presence of [^{35}S]UTP. The 550-base rat NT-3 cRNA is complementary to 392 bases of the mature rat NT-3 coding region, whereas the 540-base BDNF cRNA includes 384 bases complementary to the rat BDNF mRNA coding region [11,21]. Hybridization was conducted at 60°C for 18–24 h with the ^{35}S-labeled cRNA at a concentration of 1×10^6 cpm/50 μl/slide. Following post-hybridization washes and ribonuclease treatment, the sections were air-dried and exposed to β-Max Hyperfilm (Amersham) for 14–18 days at room temperature for generation of film autoradiograms. After autoradiographic film development, the sections were dipped in NTB2 nuclear track emulsion (Kodak; 1:1 in H_2O), air-dried, and exposed in light-tight boxes at 4°C for 4–6 weeks. After autoradiographic development of the emulsion, the sections were counterstained with Cresyl violet, coverslipped in D.P.X. mounting medium (Fluka), and analyzed with a Nikon Optiphot-2 microscope equipped with brightfield and darkfield optics. Cells were considered labeled if the density of reduced silver grains overlying the perikarya was at least 10-fold greater than background. Control sections that had been treated with ribonuclease A (45°C for 30 min) before hybridization or processed for hybridization with appropriate sense-strand riboprobes (see [11]) were devoid of specific labeling.

Film autoradiograms were analyzed with Image 1.50 software (NIH) to compare the density of hybridization for the neurotrophin mRNAs in various hippocampal subfields (dentate gyrus, CA1 and CA3) after sham treatment to that found after the various survival periods following lateral FP injury. Three to seven sections were analyzed per animal. All measurements are expressed as the mean ± S.E.M values. The data sets were compared using a two-way analysis of variance (ANOVA) for side (ipsilateral and contralateral to the injury) and groups (5 injury survival times and sham treatment). Newman-Keuls post-hoc analyses were used for pairwise comparisons with a significance level set at $P < 0.05$. BDNF and NT-3 mRNA levels did not differ by side for any of the hippocampal regions investigated, nor was there a side by group interaction (data not shown). Therefore, the hybridization data from the ipsilateral and contralateral sides were combined. The mean value of the sham control (3 h survival) was also compared to additional sham treatment animals with survival periods of 1, 6, 24, and 72 h ($n = 3$/group) post-surgery. No significant differences were found in hybridization densities among sham treatment groups with various survival periods for either BDNF or NT-3 mRNAs in any of the hippocampal subfields (data not shown).

Hybridization for BDNF mRNA was present in the granule cell layer of the dentate gyrus (stratum granulosum) and in regions CA1-CA3 of the hippocampus in the control (sham treatment) animals (Fig. 1A), similar to previous reports in normal, uninjured rats [7,21]. Unilateral FP injury resulted in a marked bilateral increase in the expression of BDNF mRNA in the dentate gyrus granule cell layer, which peaked at 3 h and remained above control levels for up to 72 h after injury (Fig. 1B–D). Densitometric measurements of film autoradiograms demonstrated that BDNF mRNA hybridization was significantly increased in the granule cell layer at all post-injury time points ($P < 0.001$; Fig. 2A). Expression of BDNF mRNA was also significantly elevated bilaterally in the hippocampal CA3 region at 1, 3 and 6 h after FP injury ($P < 0.001$), but returned to control levels by 24 h (Figs. 1 and 2B). Again, the most pronounced increase in labeling in the

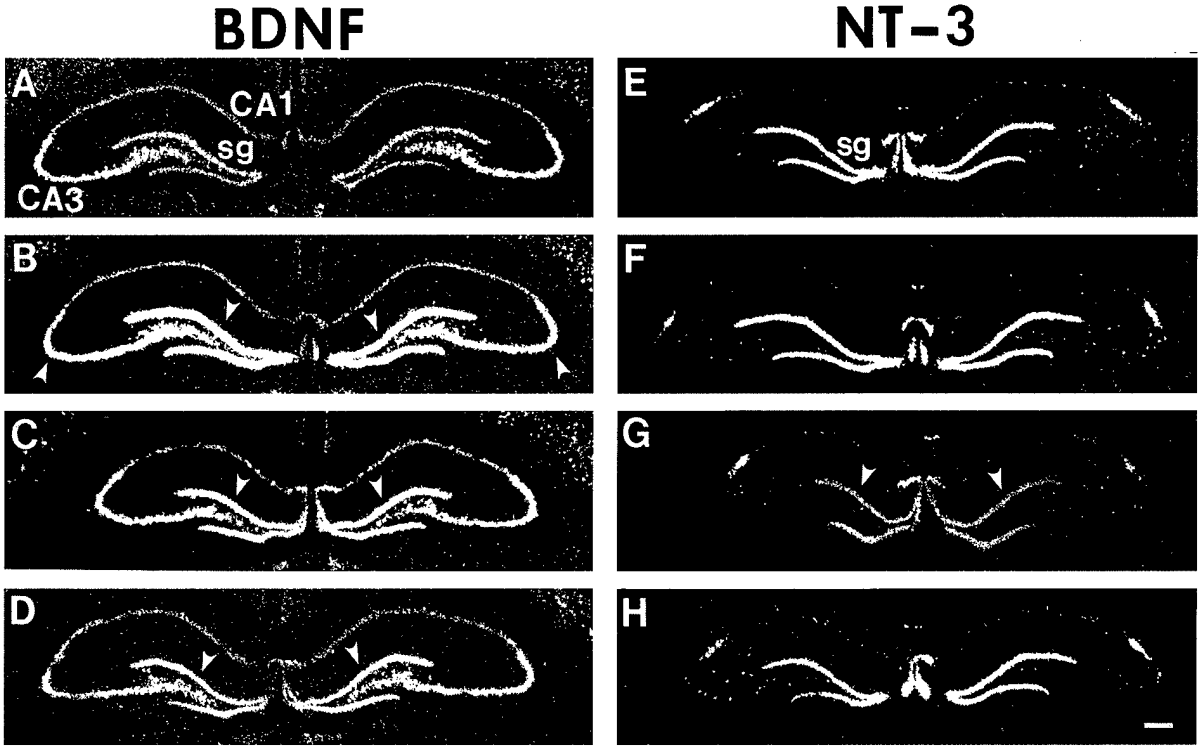

Fig. 1. Prints of film autoradiograms showing expression of BDNF (A–D) and NT-3 (E–H) mRNAs in coronal sections from control (sham) rats (A,E), and from rats subjected to moderate unilateral FP brain injury with 3 (B,F), 24 (C,G), and 72 h (D,H) survival periods. Note the increased hybridization for BDNF mRNA bilaterally in the dentate gyrus granule cell layer (stratum granulosum; sg) at all survival times following injury (B–D), and in the hippocampal CA3 region at the 3 h post-injury time point (B). In contrast, NT-3 mRNA levels are decreased bilaterally in the dentate gyrus at 24 h post-injury (G). Arrowheads in appropriate panels indicate the cell layers and survival times which differ significantly from the sham controls (see quantification in Figs. 2 and 3). Scale bar = 500 μm.

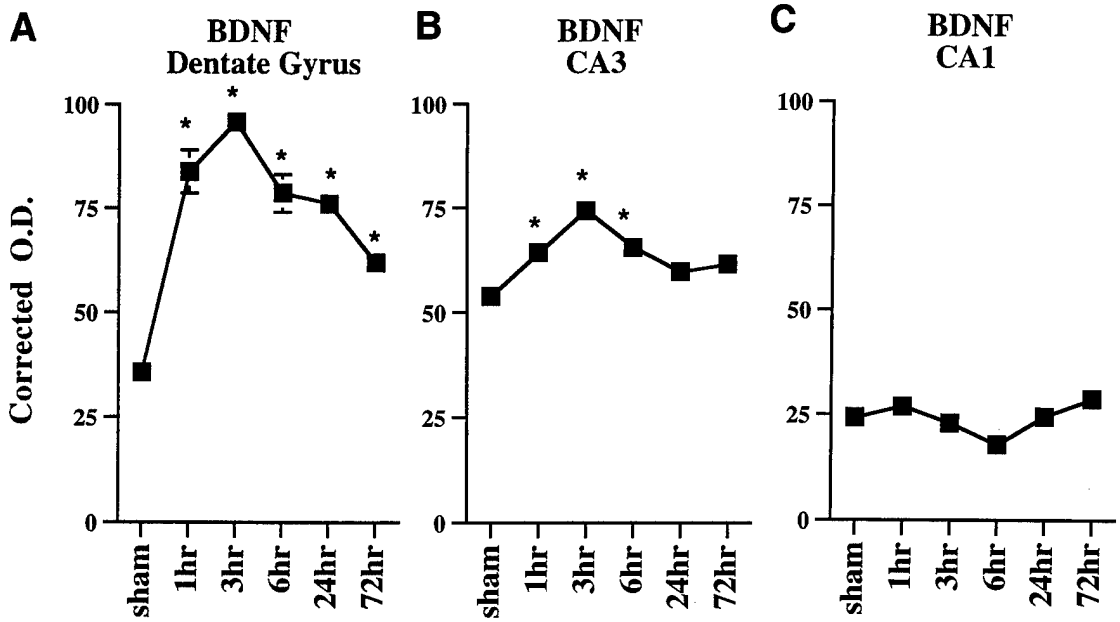

Fig. 2. Graphs showing corrected optical density (O.D.) measurements of hybridization for BDNF mRNA in the dentate gyrus stratum granulosum (A), hippocampal CA3 (B), and hippocampal CA1 (C) regions over time following lateral FP brain injury. Note the significant increase in BDNF mRNA expression in the dentate gyrus granule cell layer at all post-injury times (A), and in the hippocampal CA3 region at 1, 3, and 6 h following injury (B), compared to the sham treatment group ($^{*}P < 0.001$). Lateral FP injury did not alter BDNF mRNA levels in the hippocampal CA1 region at any of the survival times (C). Values represent mean ± S.E.M.

CA3 region was evident at 3 h after injury (Fig. 1B). No changes in expression of BDNF mRNA occurred in the CA1 region of the hippocampus following FP injury (Figs. 1 and 2C).

In the control, sham-injured animals the most prominent expression of NT-3 mRNA was localized to the dentate gyrus granule cell layer (Fig. 1E). Labeled cells were also present in regions CA2 and extreme medial CA1 of the hippocampal pyramidal cell layer, as well as infrequently scattered throughout the dentate gyrus hilus and hippocampal molecular layers (Fig. 1E). This distribution is in good agreement with previous descriptions in normal rats [7,13]. Following unilateral FP injury, hybridization for NT-3 mRNA was decreased bilaterally in the dentate gyrus granule cell layer at the 6 and 24 h survival times (Fig. 1G). By 72 h post-injury, hybridization levels had returned to near-control (sham injury) levels (Fig. 1H). Quantitative measurements of film autoradiograms confirmed that NT-3 mRNA expression was significantly reduced in the granule cells at both 6 and 24 h after FP injury ($P < 0.001$; Fig. 3), compared to sham controls. Although not analyzed densitometrically, visual examination of NT-3 mRNA hybridization in CA2 and medial CA1 indicated no apparent change in expression at any of the survival times post-injury, compared to sham controls.

The present results demonstrate that FP brain injury induces pronounced alterations in the expression of neurotrophin mRNAs in the hippocampus. Levels of BDNF mRNA were substantially increased post-injury in both the dentate gyrus granule cell and CA3 pyramidal cell layers. In contrast, expression of NT-3 mRNA was transiently decreased in the dentate gyrus, and the response was delayed relative to the early change in BDNF. Thus, traumatic brain injury differentially modulates neurotrophin gene expression in the hippocampus, in patterns and directions similar to findings in other brain injury paradigms including ischemia and seizures [12,13,27,28]. Although the present study focused on the hippocampal formation, it should be noted that obvious alterations in neurotrophin expression following FP injury were also observed in other brain regions, including the cortical lesion site, adjacent neocortical areas, the piriform cortex, and several medial thalamic nuclei (data not shown).

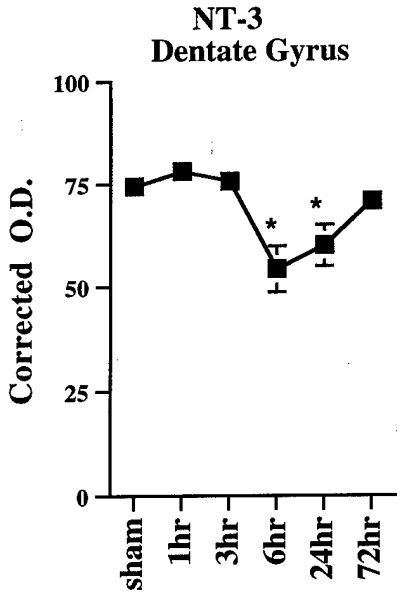

Fig. 3. Graph showing corrected optical density (O.D.) measurements of NT-3 mRNA hybridization in the dentate gyrus granule cell layer over time following lateral FP brain injury. Note the significant decline in NT-3 mRNA expression at 6 and 24 h after injury compared to the sham treatment group ($^{*}P < 0.001$). Values represent mean ± S.E.M.

Our results are consistent with recent data on the acute modulation of neurotrophin gene expression obtained with another model of traumatic brain injury, the cortical contusion impact model [48]. That study reported an increase in BDNF, but no change in NT-3, mRNA levels in the dentate gyrus granule and hippocampal pyramidal cell layers at 1, 3, and 5 h (the longest survival time examined) post-injury. The lack of change in NT-3 mRNA expression may reflect the acute time course of their study, since in the present study the decrease in NT-3 expression was not evident until 6 h after FP injury. In any event, it is now apparent from two different paradigms that a consistent response of hippocampal neurons to traumatic brain injury is dramatic, differential regulation of neurotrophin expression.

The bilateral alterations in BDNF and NT-3 expression are in contrast to the gross morphological and histological damage which has been primarily identified in hippocampal regions ipsilateral to the impact site [4,20]. However, they are consistent with more subtle changes, such as the bilateral loss of hilar neurons [30] and bilateral alterations in the expression of immediate-early genes and tumor necrosis factor-α [9,39], which have been observed following unilateral FP injury. Whereas no evidence of abnormal behavior or overt seizure activity was noted in any of the experimental groups in this study, nor in a previous study with FP injury of this severity [30], it is possible that post-traumatic subclinical seizures contributed to the alterations in expression of BDNF and NT-3. However, neurochemical changes that would be expected to occur bilaterally following seizures have only been observed unilaterally following FP brain injury [6,36,38].

The significance of the alterations in BDNF and NT-3 expression on cell survival following lateral FP injury is unclear. The dentate gyrus showed the greatest increase in BDNF compared to control values and cells in this region are selectively resistant to cell death following lateral FP injury [4,20,30]. However, BDNF expression was also elevated bilaterally in the hippocampal CA3 region, which contains numerous injured neurons on the side ipsilateral to the impact [4,20]. Numerous previous studies have supported the hypothesis that BDNF is neuroprotective following injury [1,2,17,27,28,46], whereas others have found no trophic effect [40] or an actual increase in

neuronal death [24] with BDNF treatment. Although further studies are necessary to clarify the role of BDNF following injury, one hypothesis is that it is the *amount* of BDNF available that is critical for promoting cell survival. The functional consequences of the concurrent decrease in NT-3 expression in the same cells (stratum granulosum) marked by the BDNF increase, also remain unknown. It is possible that whereas optimal neurotrophin levels may promote survival, insufficient or excessive levels may exacerbate neuronal loss. Moreover, injury-induced alterations in levels and functional states of appropriate neurotrophin receptors may also be important determinants of resulting neurotrophic functions.

In conclusion, lateral FP brain injury differentially modulates expression of BDNF and NT-3 in hippocampal neurons. These alterations are consistent with the hypothesis that widespread secondary events, including neurotrophin plasticity, occur following traumatic brain injury. Further investigations are necessary to evaluate the role of these neurotrophic factors on cell survival after experimental brain trauma.

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Expression of TrkB and TrkC mRNA is Altered in Rat Hippocampus After Experimental Brain Trauma

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Abstract

Alterations in trkB and trkC mRNA levels were investigated in the hippocampus with in situ hybridization in a rat model of traumatic brain injury. trkB, the high affinity receptor for BDNF and NT-4, mRNA was increased between 3 and 6 h, and trkC, the high affinity receptor for NT-3, mRNA was decreased at 24 h bilaterally in the dentate gyrus following a lateral fluid percussion (FP) brain injury. No time-dependent alterations were observed in the hippocampal subfields CA1 and CA3 for either of these trk mRNAs. These data demonstrate that lateral FP injury induces differential expression of trkB and trkC mRNA in the hippocampus and support a role for BDNF/trkB signal transduction in secondary events associated with traumatic brain injury.

Keywords: Traumatic brain injury, Lateral fluid percussion, Neurotrophin receptors, In situ hybridization, Neuronal plasticity

The trophic properties of neurotrophins (NTFs) are mediated through interaction with their high affinity receptors, which are transmembrane protein-tyrosine kinases (trks) (Barbacid, 1994; Jing et al., 1992; Lindsay, 1994). Activation of trk receptors involves ligand binding by the neurotrophin, receptor dimerization, autophosphorylation, and activation of tyrosine residues on various intracellular substrates (Jing et al., 1992). Under normal developmental and mature conditions, these intracellular substrates serve as signals for survival, proliferation, differentiation, and synaptogenensis, as well as other forms of neural plasticity (Lindhoml et al., 1994; Snider, 1994). Following injury, NTF/trk interactions have been linked to neuroprotection and recovery of function (Mattson & Scheff, 1994; Mochetti & Wrathall, 1995).

The widely expressed trkB protein is the high affinity receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) (Barbacid, 1994; Lindsay, 1994). BDNF can bind with any of the multiple isoforms of trkB, which include a full length receptor, and two truncated forms (Klein et al., 1990; Middlemas et al., 1991). BDNF signal transduction was originally believed to be mediated only through the full length receptor, because it alone contains the intracellular tyrosine-kinase domain (Berkemeier, et al., 1991). However, a recent study suggests that each isoform, including the truncated versions, may be capable of initiating signal transduction pathways (Baxter et al., 1997). trkB is present primarily within neurons, where it is localized to axons, dendrites and cell somata (Fryer et al., 1996). trkC is also widely expressed and is the high affinity receptor for neurotrophin-3 (NT-3) (Lamballe et al., 1991; Barbacid, 1994). There are several isoforms of trkC, some with and some without a catalytic tyrosine kinase domain (Barbacid, 1994).

Previous investigations into the role of NTFs in experimental brain trauma demonstrated that BDNF mRNA was elevated in the hippocampus as early as 1 h and up to 72 h after a lateral fluid percussion (FP) brain injury (Hicks et al., 1997). Conversely, NT-3 mRNA decreased in the hippocampus following a lateral FP brain injury (Hicks et

al., 1997). To further investigate the role of NTF/trk interactions in traumatic brain injury, we examined the acute alterations in trkB and trkC mRNA in the hippocampus following lateral (FP) brain injury in rats.

Male Sprague-Dawley rats (325-350g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) 10 min after receiving 0.15 ml of atropine (0.4 mg/ml, i.m.), and placed in a stereotaxic frame. The scalp and temporal muscles were reflected, and a stainless-steel screw was secured to the skull 1 mm anterior to bregma. A craniotomy with a 5 mm diameter was centered between bregma and lambda, 3 mm lateral to the sagittal suture. A Luer-loc hub was rigidly fixed with dental cement to the craniotomy. Experimental brain injury of moderate severity (2.0 - 2.1 atm) was induced in the anesthetized animals (n=20), using the lateral FP brain injury model. This model is well-characterized and has been previously described in detail (Cortez et al., 1989; Hicks et al., 1996; McIntosh et al., 1989). Following FP injury, rats were euthanized at 1, 3, 6, 24, or 72 h (4/survival period), in order to assess the acute response of trkB and trkC to the injury. Additional animals (n=15; 3/survival period) underwent anesthesia and surgery but were not injured (sham treatment).

After the appropriate survival times, the rats were deeply anesthetized with an overdose of sodium pentobarbital and decapitated. Brains were rapidly removed and frozen over dry ice. Tissue sections through the hippocampus were cut in the coronal plane at 10 μm in a cryostat, thaw-mounted onto Superfrost Plus (Curtin Matheson Scientific) glass slides, and stored at -20°C until processing for hybridization. Adjacent sections throughout the hippocampus of animals from the various injury and sham groups were processed for the in situ hybridization localization of mRNAs for trkB and trkC as previously described (Numan and Seroogy, 1997; Seroogy and Herman, 1997). The cRNA probes were prepared by in vitro transcription from linearized cDNA constructs with the appropriate RNA polymerase in the presence of ^{35}S-UTP. (**Kim finish this section.**)

Film autoradiograms were analyzed with Image 1.60 software (NIH) to measure the density of hybridization for the trk mRNAs in various hippocampal subfields (stratum granulosum of the dentate gyrus, strata pyramidale of CA1 and CA3). At least 3 sections were analyzed per animal. All measurements are expressed as the mean values plus or minus the standard error of the mean (SEM). The effects of treatment, survival time, and the interaction were analyzed with a two-way analysis of variance (ANOVA) in each hippocampal subfield for the side of the brain ipsilateral to the injury, contralateral to the injury, and for the bilateral sham data. Bonferonni post-hoc analyses were used for pairwise comparisons with a significance set at $P < 0.05$.

Hybridization for trkB and trkC mRNA was present in the granule cell layer of the dentate gyrus (stratum granulosum) and in regions CA1 and CA3 of the hippocampus in the control (sham treatment) animals (Fig. 1A,B), similar to previous reports in uninjured rats (Fryer et al., 1996; Klein et al., 1990; Middlemas et al., 1991). In each hippocampal subfield, sham trkB and trkC mRNA expression remained stable over time. However, following FP injury, time-dependent alterations in trkB mRNA were evident in the dentate gyrus at 3 and 6 h post-FP injury, but not at 1, 24 or 72 h (Fig. 1C,E,G). Densitometric measurements of film autoradiograms confirmed that trkB mRNA was significantly increased in both the ipsilateral and contralateral stratum granulosum of FP injured animals compared to sham controls at 3 h ($P< 0.005$; ipsilateral and contralateral both increased 42%) and at 6 h ($P < 0.05$; ipsilateral increased 28%, contralateral increased 38%), (Fig. 2A). In the strata pyramidale subfields, CA1 and CA3, time-dependent changes in trkB mRNA were not observed. However, group (ipsilateral injured vs. sham) differences were present, with a small but significant decrease in trkB mRNA in the ipsilateral CA1 region ($P < 0.005$; 11% decrease) and in the ipsilateral CA3 region ($P < 0.001$; 15% decrease) compared to shams (Fig. 2B).

Hybridization for trkC mRNA was decreased bilaterally in the dentate gyrus granule cell layer at the 24 h survival time following FP injury compared to the 24 h sham controls

(Fig. 1B,F). This decrease was not observed at earlier times or for the 72 h survival period (Fig. 1D,H). Quantitative measurements of film autoradiograms confirmed that trkC expression was significantly reduced in the stratum granulosum at 24 h after FP injury ($P< 0.02$; ipsilateral decreased 16%, contralateral decreased 12%), compared to sham controls (Fig. 3A). Time-dependent alterations in trkC mRNA were not observed in the CA1 and CA3 subfields, however group (ipsilateral injured vs. sham) differences were present. FP injury induced small but significant decreases in trkC mRNA in the ipsilateral CA1 ($P < 0.005$; 6% decrease) and ipsilateral CA3 ($P < 0.001$; 9% decrease) subfields compared to sham controls (Fig. 3B).

The major findings of this study are that trkB mRNA was significantly increased at 3 h and 6 h, and trkC mRNA was significantly decreased at 24 h bilaterally in the dentate gyrus following lateral FP brain injury. The alterations in trkB and trkC mRNA that we observed in the dentate gyrus are in general agreement with previous investigations utilizing other models of CNS injury. Induction of seizures following kindling caused a rapid and transient elevation in trkB mRNA, which peaked at 30 min and tapered off to near control values by 4 h (Merlio et al., 1993). However, in contrast to our findings, no changes were observed in trkC mRNA after seizures (Merlio et al., 1993). An ischemic insult caused an increase in trkB mRNA in the dentate gyrus at 2 h post-injury (Merlio et al., 1993), which is very similar to the temporal profile that we observed after FP injury. A similar time course was also observed after a focal, mechanical injury to one side of the brain, which produced a unilateral increase in both trkB and trkC mRNA in the dentate gyrus between 2-4 hr post-injury (Mudo et al., 1993). In contrast to Mudo et als. study, we observed a significant decrease in trkC mRNA and the alterations in the dentate gyrus were bilateral following FP injury. Recent studies have demonstrated that subtle neurological sequelae are often present bilaterally following a lateral FP injury (Hicks et al., 1997; Raghupathi et al., 1996; Fan et al., 1996), whereas overt histopathological changes are primarily restricted to

the side of the brain ipsilateral to the impact site (Cortez et al., 1989; McIntosh et al., 1989; Hicks et al., 1996; Soares et al., 1995).

A previous study demonstrated that BDNF mRNA increased to 174-235% of sham values between 1 and 72 h after FP injury, and NT-3 mRNA decreased to 73-81% of sham levels between 6 and 24 h after FP injury (Hicks et al., 1997). The increase in trkB mRNA and the decrease in trkC mRNA in the dentate gyrus after FP injury were delayed and were much less robust compared to their respective ligands. Nevertheless, the complementary increases in BDNF and trkB mRNAs suggests that this signal transduction pathway may be greatly enhanced in the dentate gyrus during the acute periods following FP injury. This is an important observation because chronic exposure to BDNF has been shown to down-regulate trkB mRNA and protein in vivo (Frank et al., 1996; Knusel et al., 1997).

The functional significance of the increase in BDNF/trkB mRNA and the decrease in NT-3/trkC mRNA in the dentate gyrus following FP injury is unknown. Originally NTF/trk interactions were believed to be important for selective neuronal survival (Alcantara et al., 1997; Johnson and Oppenheim, 1994), and this role may also be important following FP injury. BDNF/trkB signal transduction may have a neuroprotective effect on the granule cells of the dentate gyrus following FP injury, as these cells are selectively resistant to death (Cortez et al., 1989; Hicks et al., 1996; Lowenstein et al., 1992). However, CA1 cells are also resistant to lateral FP-induced degeneration, and increases in BDNF/trkB were not observed in this region of the hippocampus (Hicks et al., 1996). NT-3/trkC signal transduction has also been found to have neuroprotective effects in some models of neuronal injury (Lindsay, 1996), but that does not appear to be the case following FP injury as both neurotrophin (Hicks et al., 1997) and receptor mRNA levels were decreased in the dentate gyrus.

BDNF and trkB have also been associated with synaptogenesis and neural plasticity (Cabelli et al., 1997), and thus may be important for recovery of function following injury. Blocking trkB receptors interfered with the normal development of ocular dominance

columns in the visual cortex (Cabelli et al., 1997). Removal of facial vibrassae in mice during development resulted in a decrease in BDNF mRNA in the corresponding cortical barrel region, but an increase in the contralateral barrel region (Singh et al., 1997). A recent paper proposes that BDNF and NT-3 may have antagonistic actions on dendritic growth in cortical neurons (McAllister et al., 1997). If a similar relationship exists in the hippocampus, then the decrease in NT-3/trkC mRNA following FP injury may further amplify the effects of BDNF/trkB signal transduction on neural plasticity.

In summary, our results demonstrate that increases in trkB and decreases in trkC mRNA occur in the bilateral dentate gyrus following lateral FP injury. These data are consistent with previous studies that have shown similar alterations in BDNF and NT-3 mRNAs in this same region of the hippocampal formation. The differential regulation of BDNF/trkB and NT-3/trkC mRNAs may be important for neuronal survival or neural plasticity following traumatic brain injury.

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